Supplemental Figures

Figure S1

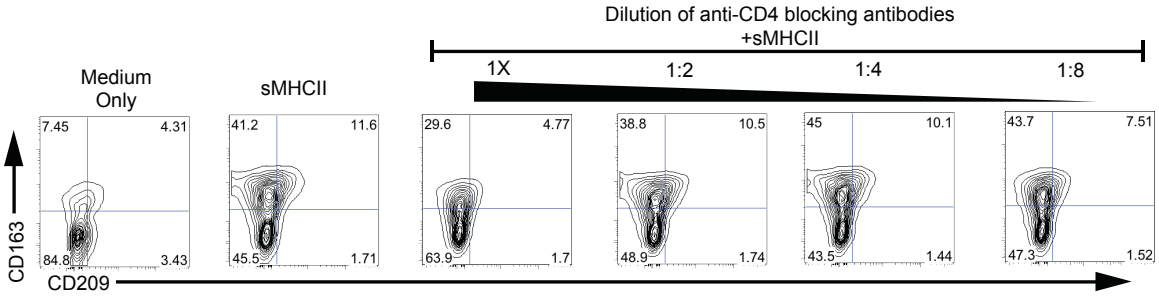

Figure S1. Inhibition of CD4:sMHC II interaction by anti-CD4-blocking antibodies occurs in a concentration dependent manner. Freshly purified monocytes were treated with medium only, sMHC II, or were preincubated for 30 minutes with 1 µg/ml (1X), 0.5 µg/ml (1:2), 0.25 µg/ml (1:4), or 0.0125 µg/ml (1:8), of each of the monoclonal anti-CD4 blocking antibodies. Preincubated cells were then treated with sMHC II. Cells were allowed to differentiate for 2 days and CD163 and CD209 expression was analyzed by flow cytometry. Dilution of the blocking antibodies decreased the specific blocking of the interaction of CD4 and sMHC II.

Figure S2

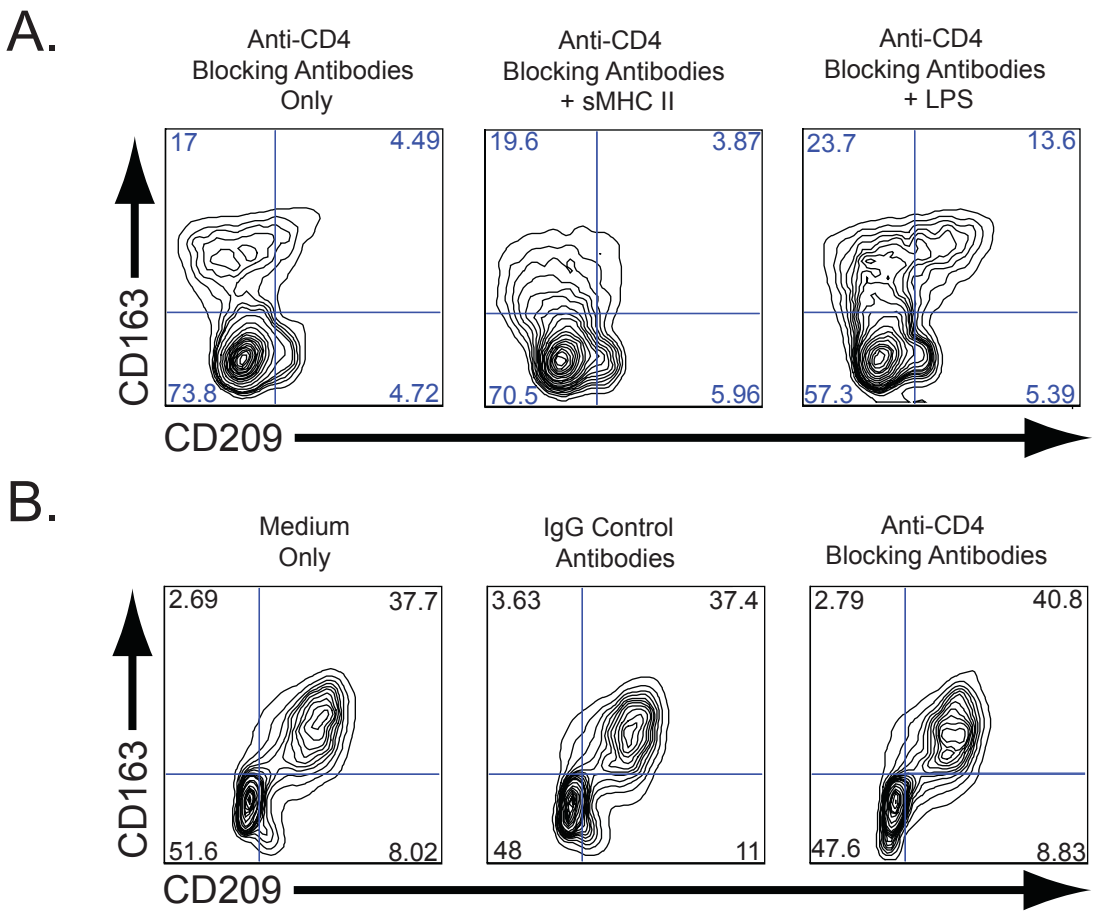

Figure S2. Specificity of CD4:MHC II interaction and effects of CD4 blocking mAbs on cellular differentiation. (A) Treatment with blocking antibodies specifically inhibits CD4:MHC II interaction and stimulation. Monocytes were pretreated with the cocktail of anti-CD4 blocking antibodies and then stimulated with either medium alone (left panel), sMHC II (middle panel), or with LPS (right). Cells stimulated with medium alone or with sMHC II showed minimal macrophage development while cells treated with LPS displayed increased macrophage development while CD4 was blocked with mAbs. (B) Treatment with CD4 blocking mAbs following stimulation does not alter macrophage development. Cells were stimulated with LPS for two days and then treated with medium alone (left panel), mouse IgG control mAbs, or with anti-CD4 blocking mAb cocktail and cultured two additional days. The data indicates that treatment of cells with CD4 blocking antibodies or with mouse IgG does not alter cellular phenotypic differentiation.

Figure S3

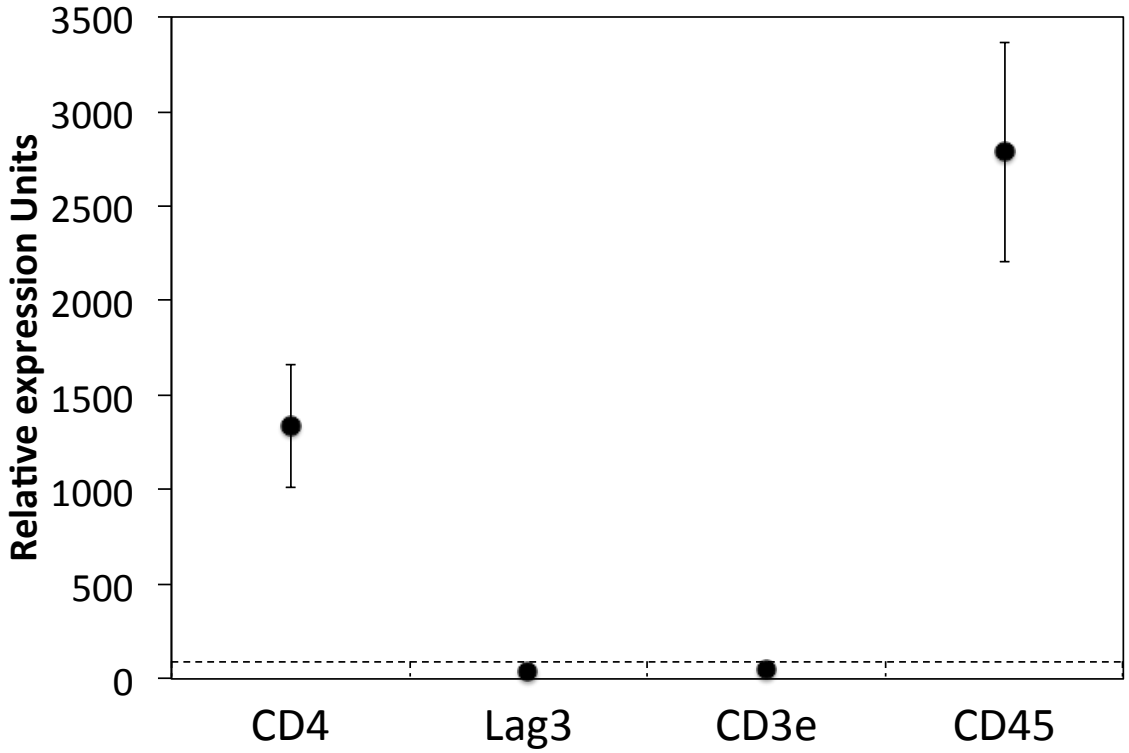

Figure S3. Lack of expression of the MHC II ligand of lymphocyte activation gene-3 (Lag-3) in primary human peripheral blood monocytes. Analysis for expression of RNA for the Lag-3 gene was performed by DNA microarray and the relative expression of the CD4, CD3, Lag-3, and CD45 genes are provided (n=5 separate donors). Error bars represent the standard deviation of the mean. The dotted line indicates the limit of detection. Expression of CD45 and CD4 were relatively high, correlating with their cell surface staining. The lack of CD3 demonstrates the efficiency of cell purification by negative selection and the lack of Lag-3 expression further indicates that MHC II is interacting with CD4 expressed on monocytes.

Figure S4

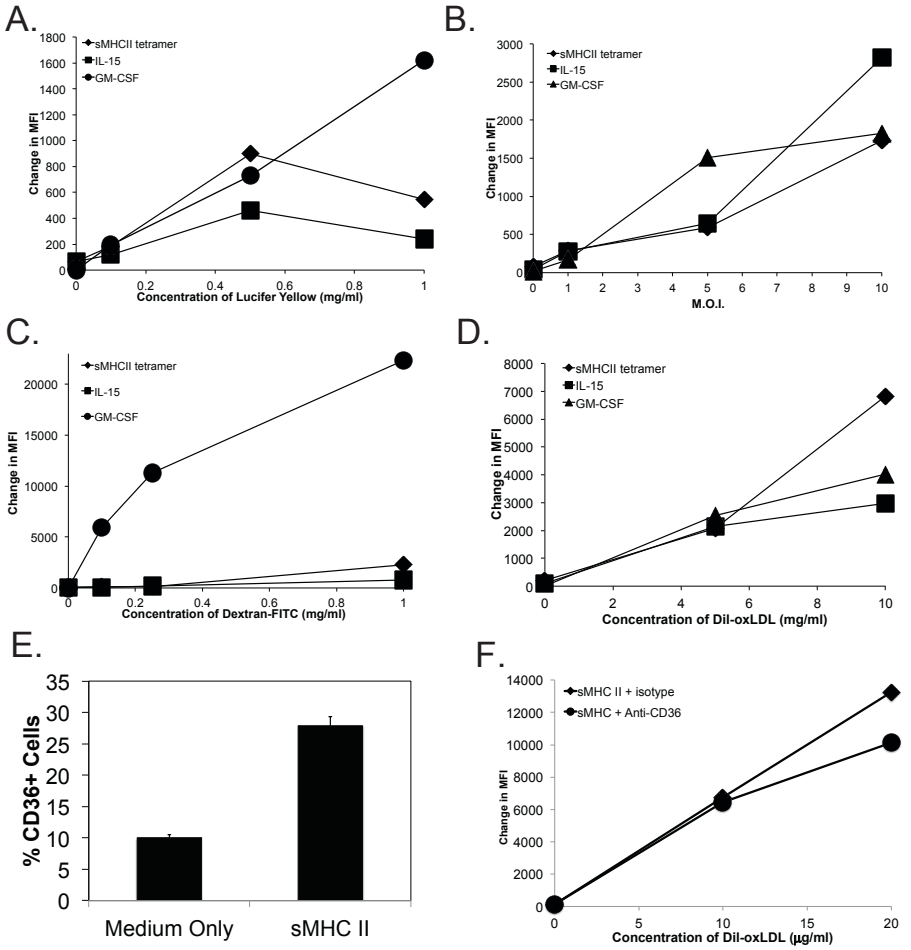

Figure S4. MHC II ligation of CD4 on human monocytes differentiates them into functional macrophages (second representative experiment). Due to the technical nature of the phagocytosis experiments, inherent assay variability including differences in fluorescent intensities of reagents on different days (which are controlled for on a given day), and limitations in cell numbers, shown is a second representative experiment utilizing freshly purified human monocytes from a different donor. As described with Figure 2, monocytes were differentiated for two days in the presence of medium only, sMHC II, IL-15, or GM-CSF and assessed for phagocytic activity, separately, of (A) Lucifer Yellow, (B) fluorescent E. coli, (C) Dextran-FITC, and (D) DiI-oxLDL at the indicated concentrations. Graphs depict the change in the mean fluorescent intensity (MFI) over cells differentiated in the presence of medium only. (E) CD36 expression levels on CD209+ macrophages differentiated for two days in medium alone (left) or with sMHC II, performed in triplicate. Error bars represent the standard deviation of the mean and differences were statistically significant ($p<0.05$, t test). (F) DiI-oxLDL uptake was assayed by macrophages differentiated for two days in sMHC II or medium only and pretreated with either mouse isotype or with anti-CD36 blocking antibody. The graph represents the change in the mean fluorescent intensity (MFI) over gated CD209+ cells differentiated in the presence of medium only.